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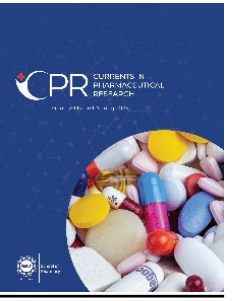

Title:	**Phytochemical Screening and Anti-dandruff Activity of Fruit Husk Extracts of *Cassia fistula* Lin**
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Phytochemical Screening and Anti-dandruff Activity of Fruit Husk Extracts of *Cassia fistula* Lin

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ABSTRACT

Medicinal plants play a substantial role in treating various disorders. This study aims to evaluate the phytochemicals and anti-dandruff activity of a fruit husk of *Cassia fistula* Linn as a remedy for treating dandruff. Various extracts of fruit husk were extracted using sequential extraction methods, namely n-hexane, chloroform, and methanol. Primarily, the polyphenols and flavonoids of the various extracts were determined. The methanol extract contained the maximum number of polyphenols (48.97 ± 0.27), while the flavonoids were found in a large amount in the n-hexane extract (123.0 ± 0.82). Anti-dandruff activity was performed using a well-diffusion method against *Malassezia* species. At 1.0 g/mL concentration, n-hexane showed a zone of inhibition of 12.5 mm, while 18.50 mm and 20.50 mm at concentrations of 1.5 g/mL and 2.0 g/mL, respectively. At the concentration of 3000 mg/mL, the inhibition zone of 23.60 mm was observed. Extracts of *Cassia fistula linn* were effective for the treatment of dandruff. Therefore, *in vivo* studies are crucially required to explore the mechanism of action.

Keywords: antimicrobials, anti-dandruff activity, *Cassia fistula*, medicinal plants, phytochemicals.

1.INTRODUCTION

Natural products are being used for the treatment of different diseases due to their therapeutic properties [1]. However, researchers have claimed that natural products are not enough to decrease the mortality and morbidity

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rate [2]. World Health Organization (WHO) has reported that 80% of people are still considering plants as a major source of medicines [3]. It is documented that people throughout the world are satisfied with traditional medicines; therefore, they prefer plant-based products over alternative medicines. Different herbal products are being used for the manufacturing of new alternative medicines because these products are efficient, effective as well as less harmful to humans and the environment [4].

Cassia fistula is one of the frequently used herbs for various types of skin diseases, and multisite studies have suggested that its use in the treatment of diabetes, fungal infections, pruritus, leukoderma, ulcers, impetigo, and hematemesis [5–8]. Dandruff is one of the most common skin disorders that not only destroys human skin but also hair cells [9]. Dandruff has recently received much attention, as it causes hair fall, loss of self-esteem, loss of immunity, and a negative image in society that is caused by *Malassezia* species that grow on greasy and oily skin [10]. The causes of dandruff include both environmental and social factors. The general method of dandruff formation reported that it is formed by the oily secretion of sebaceous glands, sebum contains lipids content that is metabolized by lipase enzymes, and triglycerides release byproduct oleic acid [11]. This by-product and a metabolic by-product of skin microorganisms penetrate the skin uppermost layer, called stratum corneum, and trigger inflammatory responses and cause disturbance in the internal hemostatic environment [11]. This results in cleavage of cells of the stratum corneum that causes several scalp disorders. Naturally derived drugs are preferable over allopathic drugs for the treatment of infectious diseases because they have maximum therapeutic effects with minimum side effects. Multisite studies reported that various plant species are used for the treatment of dandruff [10, 12, 13]. The existing literature review of *Cassia fistula* reported the presence of many bioactive chemical constituents that possess pharmacological activities [14].Various biological activities have been performed on various parts of plant [2, 15, 16]; however, the fruit-husk has received less attention. Therefore, the main objective of this study is to evaluate the *in vitro* anti-dandruff activity of various extracts of fruit husk of *Cassia fistula* Lin.

2.MATERIALS AND METHODS

2.1. Chemical and Solvents

Acetone (Merck, Germany), Aluminium nitrate (Merck, Germany), Chloroform (Merck, Germany), Copper sulphate (Merck, Germany), Gallic acid (Sinochem, China), Glucose (Merck, Germany), Methanol (Merck, Germany), n-hexane (Merck, Germany), Potassium tartrate (Merck, Germany), Quercetin (Sigma Life Sciences, Germany), sodium carbonate (Merck, Germany). Sabouraud's agar (SDA) media, olive oil, dimethyl sulfoxide (Merck, Germany).

2.2. Instruments

Electric weighing balance (BL, 2005, Setra, USA), Ultraviolet-visible (UV-Vis) spectrophotometer (Model-2500 Shimadzu Scientific Instrumentation, USA.), Vortex mixer (SLV-6, Seoulin Biosciences, Korea). Laboratory hot air oven (TYPHIZ, Suszukara Prozinowa, Poland), Ultrasonic mixer (DSA50-CKI-1.8L), Incubator (MIR-153 Sanyo Electric Corporation Japan).

2.3.Collection of Plant Material

In January 2020, the fruit *Cassia fistula* was purchased from Akbari Mandi, Lahore, Pakistan. This plant was authenticated by taxonomist Prof. Dr. Zaheer-ur-Din Khan, Department of Botany, Government College University, Lahore, Pakistan. The voucher specimen (GC Herb. Bot. 3760) was deposited in the herbarium of GCU. The fruit husk was then separated, washed, cleaned, and dried for 20 days under the shade at room temperature. The fruit husk was crushed into fine powders and then stored in an air-tight bag.

2.4. Extraction of Plant Material

Various solvents were used in the extraction process to extract plant constituents. 50 g of plant material was taken, and the desired content was separated with the help of a sequential extraction method. Soxhlet apparatus was used for the extraction of the desired content. The solvents used were n-hexane, chloroform, and methanol. The material was placed in the thimble of Soxhlet apparatus and heated until colorless material was obtained. Each extract was then collected and dried with the help of a rotary evaporator at 60°C. The extracts were reserved in the refrigerator at 4°C.

2.5. Estimation of Polyphenols

The Slinkard et al. [17] conducted a study to describe the procedure of estimation of total amount of phenol in the *Cassia fistula* fruit husk powder. In this procedure, gallic acid was used as the standard to draw the standard calibration curve. In this procedure the sample solution and stock standard solution were prepared in the methanol. In this procedure the stock solution 10, 20, 40, and 120 µL of sample and Gallic acid was taken, respectively and distilled water was added to make up the volume of 1000 µL. A 200 µL of standard and sample solution was taken in the test tube and 0.2 mL FC reagent was added in these tubes. 1 mL of 15% Na_2CO_3 was added after 4 minutes. Though, blank was made in the same way, methanol (200 µL) was applied instead of inserting a sample. The solution was incubated for 2 hours at room temperature. Absorbance was then estimated at 760 nm.

2.6. Estimation of Flavonoids

Pavun and his colleagues conducted a study that introduced a method to determine the quantification of total flavonoids within the *Cassia fistula* [18]. Quercetin was used as a standard to plot a standard curve. Methanol was used to prepare the stock solution of both sample solution as well as the standard solution. Further 10, 20, 40, 80, and 120 µL dilutions were prepared from the stock solution. A 200 µL of normal solution and 200 µL of sample, 100 µL of 10% (w/v) of aluminum nitrate solution were added in test tubes. Afterwards, a total of 100 µL 1M potassium acetate solution and 4.6mL of distilled water were added. The test tubes were incubated for 45 minutes at room temperature. The absorbance was then measured at 415 nm wavelength.

2.7. Anti-dandruff Activity of *Cassia fistula*

2.7.1. Collection of Dandruff. The organism was isolated from scalp of persons suffering from dandruff flakes or scales, collected by partitioning the hair with a sterile comb and scrapping approximately one-inch area using a sterile scalpel. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight [8].

2.7.2. Preparation of Media. Sabouraud's dextrose agar medium was prepared by taking dextrose (20 g), peptone (10 g), agar (20 g), and sterilized corn oil (5 mL) at pH 5.6 and temperature 25 °C. The material was suspended in 1 L of distilled water. It was heated to dissolve the

medium completely and sterilized by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes.

2.7.3. Well diffusion Method. By using a well diffusion method, organic extracts were screened and then test solutions were prepared for each extract in dimethyl sulfoxide (DMSO). Fluconazole was used as positive control solution and DMSO was used as negative controls. For filamentous fungi, Sabouraud's dextrose agar (SDA) was used. Each extract solution and controls were dropped in a well of diameter 6-mm. Plates are then placed in the incubator and then incubated for 24 h at 37℃ to grow bacteria and yeast cultures for 3 days at room temperature for filamentous fungi in aerobic condition. The diameter of the inhibition zone was measured and recorded around each well. Antimicrobial activity was calculated as the ratio of the average of inhibition zones produced by the extract under test and the average of inhibition zones caused by the positive controls. Each test was performed threefold.

2.8. Statistical Analysis

All the tests were performed in triplicate and the mean were calculated. All the values were expressed as means ± standard deviation (SD).

3.RESULTS

3.1. Estimation of Polyphenols and Flavonoids

The results of quantitative analysis for the presence of polyphenols and flavonoids in various plant extracts of fruit husk of *Cassia fistula* are presented in Table 1. All extracts showed positive results for the presence of phytochemicals. The findings reported that polyphenols were present in large quantity in methanolic extract, while n-Hexane extract contained maximum number of flavonoids.

Table 1. Total content (mg/g) of Phytochemicals of Fruit Husk Extracts of *Cassia fistula linn*

Extracts	Total Polyphenols (mg/g)	Total Flavonoids (mg/g)
n-Hexane	33.2 ± 0.21	123.0 ± 0.82
Chloroform	3.0 ± 0.33	19.7 ± 0.42
Methanol	48.9 ± 0.27	3.0 ± 0.12

Table 2. Zone of inhibition (mm) of *Malassezia furfur* with various extracts of fruit husk of *Cassia fistula* Linn.

Concentration (g/mL)	Zone of inhibition (mm)			
	Fluconazole	n-Hexane	Chloroform	Methanol
1.0	14.15 ± 0.23	12.95 ± 1.89	0 ± 0	14.50 ± 0.82
1.5	18.80 ± 0.43	13.70 ± 1.65	12.50 ± 0.12	14.95 ± 2.11
2.0	22.50 ± 1.26	18.65 ± 0.76	12.80 ± 0.67	15.25 ± 0.21
3.0	23.60 ± 0.9	20.20 ± 0.45	14.35 ± 0.58	17.85 ± 0.11

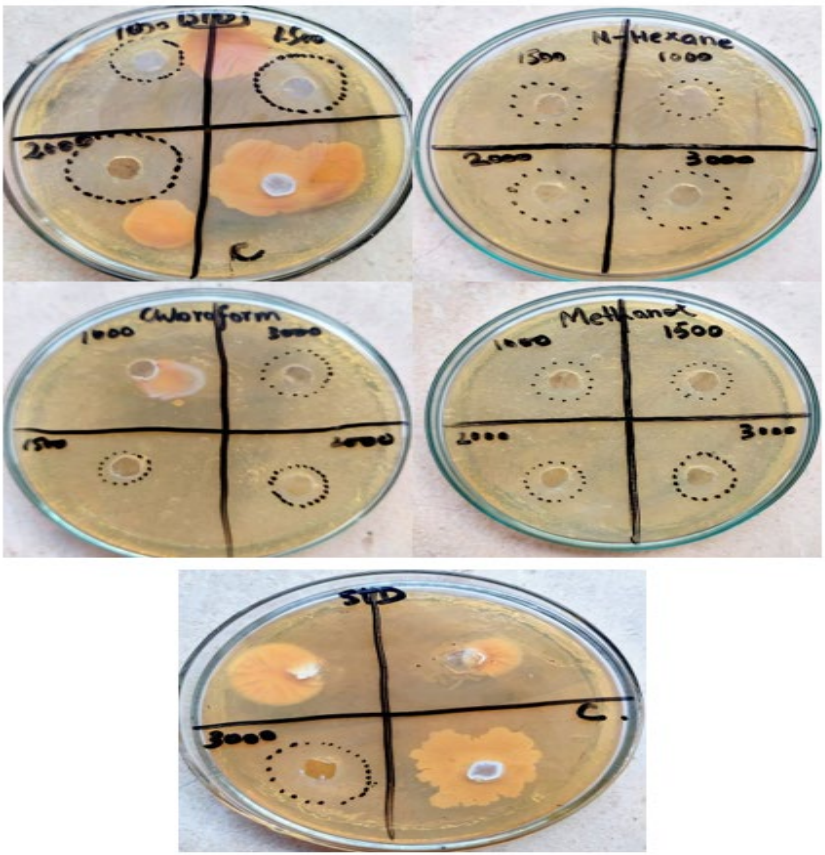

Figure 1. Anti-dandruff Activity of Various Extracts of *Cassia fistula* Linn and Fluconazole.

3.2.Estimation of Anti-Dandruff Activity

Table 2 shows that the extract obtained from the fruit husk of *Cassia fistula* was very efficacious against the tested pathogen. The extract

obtained by n-hexane solution showed a 12.95 mm inhibition zone at 1.0 g/mL concentration. While inhibition zone of 18.50 mm at 1.5 g/mL concentration was reasonably effective. At 2.0 g/mL, the inhibition zone was observed to be 25.50 mm, while 23.60 mm inhibition zone was obtained at 3.0 g/mL (PLATE-2). If chloroform solution is used to extract the active constituents from fruit husk of the plant, then at 1.0 g/mL concentration inhibition zone was not observed. However, at a concentration of 1.5 g/mL, inhibition zone was observed as 12.50 mm. Furthermore, at concentration of 2.0 g/mL, 12.80 mm inhibition zone was calculated. Similarly, at concentration of 3.0 g/mL, inhibition zone of 14.35 was noted (PLATE-3). If the methanolic extract is obtained at 1.0 g/mL concentration, then it shows an inhibition zone of 14.50 mm. Additionally, 14.95 mm inhibition zone was observed if concentration put to 1.5 g/mL. At the concentration of 2.0 g/mL, the inhibition zone was observed to be 15.25 mm. While at concentration of 3.0 g/mL, the inhibition zone was measured to be at 17.85 mm.

4.DISCUSSION

The experimental plant *Cassia fistula* is traditionally used for the treatment and management of various diseases. This research is substantial because no other research has been conducted on this topic up to the date. Therefore, this study was performed to assess the anti-dandruff activity of various extracts of *Cassia fistula* by using a well diffusion method. The extract was first passed through the phytochemical screening. Phytochemical screening helps to identify the active compounds responsible for the plant's therapeutic effects, allowing for targeted use in the treatment of specific ailments [19, 20]. Moreover, phytochemical screening is essential to the discovery of new drugs and the development of alternative therapies for various diseases. The current study reported that the methanolic extract contained the maximum number of polyphenols, followed by the n-hexane and chloroform extracts. Several factors, such as environmental factors and processing methods may [21] affect the presence of polyphenol content [22].

The *in-vitro* evaluation of the anti-dandruff activity of the fruit husk of *Cassia fistula* showed promising results, showcasing its potential as a natural remedy for this common scalp disorder. Similarly, another study reported that other species of *Cassia* are also effective against *Malassezia* species [23]. The n-hexane extract was found to be more effective than the

methanolic and chloroform extracts. The chloroform extract did not show activity at 1.0 g/mL. It may be due to their low polyphenolic content because polyphenols have received much attention in the realm of drug discovery, specifically in the search for anti-dandruff drugs [21]. The observed inhibition of microbial growth at different concentrations aligns with the known antimicrobial properties of anthraquinones present in *Cassia fistula* [24, 25]. However, *Cassia alata* and *Cassia auriculata* have the potential to treat dandruff at low concentrations [8]. This study suggests a potential role in countering the overgrowth of *Malassezia* species, an important factor in the pathogenesis of dandruff [26]. Moreover, the comparative analysis with existing literature highlighted the potential characteristics of anti-dandruff-activity of plant [27]. *Malassezia* species of fungus bring on lipolysis that hydrolyzes triglycerides in human sebum into free fatty acids, which result in both hair loss and scalp disorders [28].

While most of the medicinal plants primarily focus on the antimicrobial effects, *Cassia fistula* appears to offer a broad spectrum, addressing not only fungal overgrowth but also regulates the sebum. The transition from *in vitro* to *in vivo* studies and subsequent clinical trials are crucial to validate these findings and ascertain the practical applicability of *Cassia fistula* in the development of novel anti-dandruff formulations. Further exploration of its efficacy in real-world condition would contribute to establishing *Cassia fistula* as a viable and natural alternative for individuals seeking relief from dandruff-related issues.

4.1. Conclusion

The current study's findings reported that methanol extract contained a substantial number of polyphenols and n-hexane, which exhibited the highest flavonoids content. The current study also revealed that *Cassia fistula* has the potential to treat dandruff. However, further investigation is required to assess the mechanism of action in animal models. The positive outcomes may be due to the presence of phytochemicals; therefore, further characterization and isolation of active compounds from plant extracts are essentially required.

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